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REGIONAL ANALYSIS OF CANDIDATE GENES ASSOCIATED WITH ACUTE GVHD FOLLOWING ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Many non-MHC genetic variants that influence the risk and severity of acute graft versus host disease (GVHD) and other adverse transplant outcomes have been identified. However, the genetic regions surrounding these candidate variants have not been evaluated in detail to confirm the region of highest association and identify targets for functional studies.

Methods: We evaluated candidate genes previously reported to be associated with acute GVHD and used genome wide data generated using the Affymetrix GeneChip® Genome-Wide Human Single Nucleotide Polymorphism (SNP) Array 5.0 from a cohort of 1,560 donor-recipient pairs who had allogeneic hematopoietic cell transplantation between 1992 and 2005. Actual and imputed SNP genotypes within and +/-50k base pair surrounding each gene were evaluated. A permutation method was used to establish gene-wide statistical significance, accommodating multiple comparisons of SNPs within and surrounding each gene.

Results: 40 publications between 1998 to 2009 reported associations between acute GVHD and 34 genetic variants in 24 genes: CCR5, CD31, CTLA4, ESa, FAS, FcγRIIIb, HSP70-hom, IFNγ, IL10, IL10Rβ, IL1α, IL1β, IL1Ra, IL2, IL23R, IL6, MADCAM1, MTHFR, NOD2, TGFβ1, TNF, TNFRII, VDR, VEGFα. Fifteen of the 34 originally reported SNPs were successfully imputed or included on the 5.0 array. Among these 15, we reproduced the association of rs2075800 in HSPA1L with grade III-IV acute GVHD ($p = 0.0035$), but were not able to confirm the association between SNPs and GVHD. The SNPs that were most significantly associated with GVHD were rarely in the same location as the original candidate SNP. In adjusted analyses, four (CTLA4, IL6, MADCAM1, and TNFRII) were associated with grade II-IV acute GVHD (p-value range 0.0017-0.0085), eight were associated with grade III-IV acute GVHD (CCR5, HSPA1L, IFNG, IL10RB, MADCAM1, NOD2/CARD15, TNF, and VEGFα; p-value range 0.0005-0.007), six (IL10, IL23R, NOD2/CARD15 and VDR) were associated with transplant-related mortality (p-value range < 00001-0.0017), and six (FAS, IL10, IL23R, MADCAM1, NOD2/CARD15 and TGFB1) were associated with all cause mortality (p-value range < 0.0001-0.0051).

Conclusions: These results illustrate a high-resolution approach utilizing genome wide data for fine-mapping genetic associations identified through candidate studies and may provide important information for guiding the selection of candidates for functional studies and biomarker clinical trials.

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IN VIVO T CELL DEPLETION WITH DEXAMETHASONE REDUCES GRAFT VERSUS HOST DISEASE (GVHD) WITH MINIMAL SIDE EFFECTS AND NO ADDITIONAL RISK OF EARLY RELAPSE AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION

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Cytokine mobilized stem cells collected from peripheral blood are routinely used for allogeneic hematopoietic stem cell transplantation (alloHSCT). Advantages to PBSC use include better donor tolerance and more rapid engraftment than marrow source stem cells. Disadvantages of PBSC include the increased incidence and severity of chronic GVHD due to higher numbers of alloreactive T cells.[1,2]

Objective: We hypothesized that a short course of dexamethasone (Dex) given to donors prior to PBSC collection would reduce the T cell content of the graft resulting in decreased incidence and severity of GVHD without compromise to engraftment, infection rate, immune reconstitution or relapse rates.

Methods: At our institution, 122 consecutive patients treated with alloHSCT for various malignancies between 1998-2007 received PBSCs purged of T cells in vivo by oral administration of Dex ($10mg/m^2$) to the donor on each of the last 3 days of G-CSF mobilization. The median patient age was 48 (range 19-68), all were conditioned with busulfan based regimens, 41 patients had low risk ASBMT disease status vs 53 with high risk disease. All received CSA+MTX GVH prophylaxis.

Results: An earlier report of the first 98 patients vs 29 controls mobilized without Dex demonstrated a 2 log reduction in the $CD3^+$ graft content of the collected product, and a low incidence and severity of acute GVHD(19% grade II - IV at 100 days). We now report an updated retrospective multivariate analysis of the incidence of acute and chronic GVHD, time to engraftment, relapse rates, and 1 year overall survival of the entire cohort, with longer follow up (min = 1yr). Final analysis of all graft products contained $CD3^+$ and $CD34^+$ contents of $3.46x10^8$(sd = $2.32x10^8$) and $7.85x10^6$(sd = $5.02x10^6$) cells/kg respectively vs. $1.09x10^{10}$ $CD3^+$(sd $2.19x10^{10}$) and $7.84x10^6$ $CD34^+$ cells/kg (sd $5.34x10^6$) in the 29 institutional controls. Patients in the G-Dex group experienced 26% grade II-IV acute and 9.3% extensive chronic GVHD, uniform engraftment success, normal pattern immune reconstitution, and an 18% malignant disease relapse rate at 1 year.

Conclusion: A short course of Dex given to donors prior to PBSC collection is a viable strategy for in vivo T cell depletion. Donors tolerated the steroid well, bone pain symptoms associated with GCSF mobilization were ameliorated, and there were no untoward effects of steroid use. Comparison of this patient population with 3:1 matched CIBMTR controls is approved and in progress.

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CELLULAR ANTI-VIRAL IMMUNE RESPONSES ARE INCREASED IN THE ABSENCE OF VASOACTIVE INTESTINAL PEPTIDE

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Backgrounds: Previously we reported that mice "knocked-out" for vasoactive intestinal peptide (VIP) had increased blood levels of anti-viral T-cells following murine cytomegalovirus (mCMV) infection, and that the absence of VIP in donor cells did not increase GvHD in a murine model of allogeneic BMT. In this study, we used VIP-knock-out (KO) mice to further investigate the effects and mechanisms of VIP on murine anti-viral immune responses in peripheral lymphoid organs.

Methods: VIP-KO mice and wild-type (WT) littermates were infected with a sub-lethal dose of mCMV by intraperitoneal injection. The mice were euthanized and the spleens and the livers sampled at distinct time points. $CD8^+$ T-cell responses to virus were measured by flow cytometry using mCMV peptide-MHC class I-tetramers. Natural killer (NK) cell activity was tested by lysis of YAC-1 target cells. Viral load was determined by plaque assay. Expressions of CD25, CD62L, CD69, ICOS, killer cell lectin-like receptor G1 (KLRG1), PD-1, TNF-α, IFN-γ, IL-4, and IL-10 in lymphocyte subsets were examined by flow cytometry with respective isotype controls.

Results: VIP-KO mice had faster and higher levels of antigen specific anti-viral T-cells, enhanced NK cytolytic activity, and faster rate of viral clearance after mCMV infection compared with WT mice. VIP-KO $CD4^+$ T-cells transiently expressed higher levels of CD25 compared with WT $CD4^+$ T-cells at 3 days after mCMV infection. VIP-KO mice had equal levels of PD-1 for 10 days post infection compared with WT mice. The levels of $ICOS^+$ and $KLRG1^+$ activated $CD8^+$ T-cells were increased in mCMV-infected VIP-KO mice and their levels correlated with the enhanced mCMV-peptide-MHC $tetramer^+$ specific anti-viral responses in $CD8^+$ T-cells. IFN-γ expression was significantly higher in VIP-KO NK cells and in the subset of $CD8^+$ dendritic cells (DC), while TNF-α expression was higher in $CD4^+$ and $CD8^+$ T-cells from mCMV-infected VIP-KO mice compared with WT controls. No significant differences were noted in the levels of Th2 cytokine expression in lymphocytes between VIP-KO and WT mice after mCMV infection. Taken together, these data suggest that VIP expression by T-cells modulates DC and NK function and suppresses Th1/Tc1 cellular anti-viral immune responses.

Conclusion: VIP negatively regulates T-cell and NK cell anti-viral immune responses. The VIP pathway is an attractive target for pharmacological intervention to enhance anti-viral activity of NK cells and $CD8^+$ T-cells.